

## SIGNAL COUNTING FOR IN SITU HYBRIDIZATION

### ABSTRACT

Fluorescently tagged nucleic acid probe signals are counted in  
5 biological specimens by determining a ratio of signals from a test probe to  
signals of a reference probe. Probe signals need not be counted with  
reference to cells, nuclei, or nuclear contours. Gene amplification or deletion  
can thus be detected by analyzing the ratio. Successive image slices are  
obtained by confocal microscopy, and the images are digitized. The digital  
10 images are transformed and analyzed to combine contiguous fluorescent  
signal segments in successive optical sections to identify discrete probe  
signals, or spots. Spots overlapping in the axial and transverse dimensions of  
a three-dimensional representation of the biological specimens can be  
distinguished. A graphical user interface presents various features for  
15 consideration by a user, who can provide guidance to a computer system  
counting the spots. Various features directed to identifying spot clusters and  
autofluorescent material can increase accuracy of spot counting.